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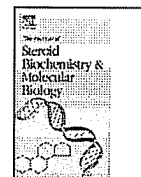
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journal homepage: www.elsevier.com/locate/jsbmbClinical applications for estetrol[☆]Monique Visser*, Herjan J.T. Coelingh Bennink¹

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ABSTRACT

In this paper the potential clinical applications for the human fetal estrogen estetrol (E₄) are presented based on recently obtained data in preclinical and clinical studies. In the past E₄ has been classified as a weak estrogen due to its rather low estrogen receptor affinity. However, recent research has demonstrated that due to its favorable pharmacokinetic properties, especially the slow elimination and long half-life, E₄ is an effective orally bioavailable estrogen agonist with estrogen antagonistic effects on the breast in the presence of estradiol. Based on the pharmacokinetic properties, the pharmacological profile and the safety and efficacy results in human studies, E₄ seems potentially suitable as a drug for human use in applications such as hormone replacement therapy (vaginal atrophy and vasomotor symptoms), contraception, osteoporosis and breast cancer.

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1. Introduction

Estetrol (E₄) is a human steroid, produced by the fetal liver during pregnancy only. This natural hormone was discovered in urine of pregnant women by Diczfalussy and coworkers in 1965 [1]. Based on its physical and chemical characteristics it was concluded that E₄ is identical with 15 α -hydroxyestriol (15 α -OHE₃) or estra-1,3,5(10)-triene-3,15 α -,16 α -,17 β -tetrol [2]. Estetrol has the structure of an estrogenic steroid with four hydroxyl groups which explains the acronym E₄. Estetrol is synthesized by the fetal liver during human pregnancy and reaches the maternal circulation through the placenta. The fetal liver is the exclusive site of 15 α - and 16 α -hydroxylation [3–5]. After birth the neonatal liver rapidly loses its capacity to synthesize E₄.

Estetrol was already detected at 9 weeks of pregnancy in maternal urine [6,7]. During the second trimester of pregnancy high levels were found in maternal plasma, with steadily rising concentrations of unconjugated E₄ to about 1 ng/mL (3 nmol/L) towards the end of pregnancy [8,9]. So far the physiological function of E₄ has not been studied and is unknown.

The possible use of E₄ as a marker for fetal well-being has been studied quite extensively. However, due to the large intra- and inter-individual variation of maternal E₄ plasma levels during pregnancy this appeared not to be feasible [10–14]. More details on the history

of E₄ and data from studies in the period from 1965 to 1984 have been summarized in two review papers [8,15].

During the last 7 years E₄ has been studied extensively. High oral absorption and bioavailability with a 2–3 h elimination half-life in the rat has been established [16]. In the human E₄ showed a high and dose-proportional oral bioavailability and a remarkably long terminal elimination half-life of about 28 h [17].

Estetrol has a moderate affinity for both human ER α and ER β receptors with a 4–5-fold preference for the ER α [18]. In addition it was found that E₄ binds highly selectively to the estrogen receptors.

In rat and human hepatocytes the rate of E₄ metabolism was studied and found to be slow [18]. In addition E₄ did not inhibit any of the major drug metabolizing cytochrome P450 enzymes CYP1A2, CYP2C9, CYP2C19, CYP2D6 and CYP3A4 at a high concentration of 10 μ M [18]. This in contrast with the effects of estradiol (E₂) and ethinylestradiol (EE) on these enzymes.

Using the HepG2 and Hep89 cell lines the ER α -dependent effect of E₂, estriol (E₃), E₄ and EE on SHBG production was investigated [19]. Estetrol did not stimulate the production of SHBG in both cell lines, while E₂, E₃ and EE all showed a dose-dependent ER α -mediated increase in SHBG production. Estradiol showed the most prominent increase in the production of SHBG, while E₃ and EE showed a lower and comparable increase in SHBG. In addition no detectable binding of E₄ was found to both the estrogenic and androgenic human SHBG steroid-binding sites [19]. Both testosterone and E₂ were bound with high affinity, whereas EE bound to SHBG with low affinity [19]. These results indicate that the plasma distribution of E₄ or its availability for target tissues may not be affected by SHBG levels in contrast to other natural steroids such as E₂ and testosterone.

Estetrol has also been studied in predictive, validated, pharmacological *in vivo* rat models and in phases I and IIA clinical trials

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in the human females. This data indicate that E_4 may be suitable for use in several indications, e.g. hormone replacement therapy (vasomotor symptoms and vaginal atrophy), contraception, osteoporosis and breast cancer. In the current paper these potential clinical applications of E_4 are reviewed based on data generated from pharmacological studies as well as clinical trials performed with E_4 .

2. Vasomotor symptoms

The efficacy of E_4 in alleviating hot flushes was studied in an experimental rat model considered representative for menopausal vasomotor symptoms [20].

In this model the thermal responses in the tail skin of morphine-dependent ovariectomised (OVX) rats was recorded after administration of naloxone (NAL). Six groups of rats were treated orally for 8 days as follows: vehicle (negative) control; E_4 : 0.1, 0.3, 1.0 and 3.0 mg/(kg day) and as active (positive) control EE: 0.3 mg/(kg day). On day 8, tail skin temperature (TST) was recorded at baseline and for 60 min at 5-min intervals following NAL administration. In control animals TST increased sharply by about 4.5 °C after NAL treatment and reverted to baseline by 60 min. Estetrol suppressed the TST increase in a dose-dependent fashion (Fig. 1). The highest dose of E_4 tested (3 mg/(kg day)) was equipotent to a 10-fold lower dose of EE. Both fully suppressed TST changes [20].

It is concluded that E_4 is effective in preventing temperature rises in an experimental animal model considered representative for studying the effect of drugs on the menopausal hot flush (vasomotor symptoms). In this model the potency of E_4 was 10-fold lower compared to EE. A phase I multiple dose study to evaluate the safety, tolerability, pharmacokinetics and pharmacodynamics of four dosages of E_4 after daily administration for 28 days was performed in healthy postmenopausal women (unpublished data). In the 2 and 10 mg/day E_4 dose groups women were included with at least 30 hot flushes per week. Estetrol was effective in alleviating hot flushes in most women in both dose groups comparable with a 2 mg/day E_2 -valerate control group during the treatment period of 4 weeks. Although the level of evidence of this study design is not high, it indicates efficacy at these dose levels.

These results suggest that E_4 may be effective for the treatment of hot flushes and other vasomotor symptoms in peri- and postmenopausal women.

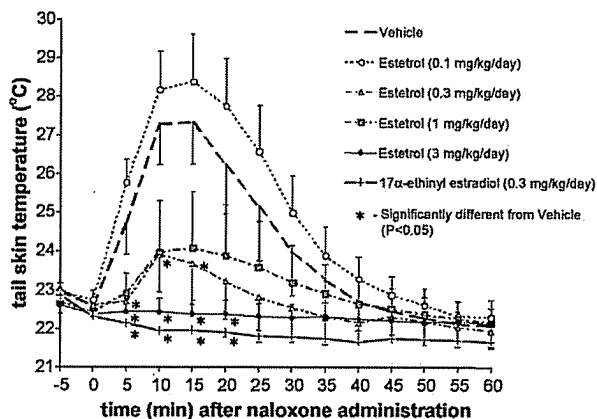


Fig. 1. The effects of estetrol and ethinylestradiol on the hot flush response induced by naloxone in female ovariectomised rats.

3. Vaginal atrophy

The effect of E_4 on vaginal cornification and uterine weight was studied in OVX rats [21]. Six groups of rats were treated orally once daily for 7 days as follows: vehicle (negative) control; E_4 : 0.1, 0.3, 1.0 and 3.0 mg/(kg day) and EE 0.05 mg/(kg day) as active (positive) control. Vaginal lavages were obtained daily and on day 7 uterine wet weight was determined. Vaginal cornification was observed by day 5 in all rats at all E_4 doses and in the animals receiving EE, but not in the control rats (Fig. 2). The onset of cornification with E_4 was dose-dependent. After 7 days treatment, the two highest E_4 doses (1.0 and 3.0 mg) induced statistically significantly higher uterine wet weight (myometrium) compared to vehicle [21]. Also a pharmacological study was performed to investigate the effect of E_4 on prevention of bone loss [16]. In this study the uterus of the OVX rats was excised after 4 weeks of treatment. Wet uterine weight (myometrium) was estimated and histological investigation of the endometrium was performed. Four weeks of E_4 treatment induced dose-dependent increases in uterine weight of OVX rats. The potency of EE in increasing uterine weight in this model was 5–25 times higher than that of E_4 .

In addition, estetrol showed a dose-dependent proliferative estrogenic effect on the rat endometrium after 4 weeks treatment [16]. The order of increasing potency per mg/(kg day) was estimated as follows: 0.1 mg E_4 < 0.5 mg E_4 < 0.1 mg EE < 2.5 mg E_4 , indicating that E_4 was less potent than EE. In summary, estrogenic activity of E_4 was demonstrated in three tissues in OVX rats; vaginal epithelium, myometrium and endometrium. The potency of E_4 was approximately 20-fold lower compared to EE.

In a phase I multiple dose study with 2, 10, 20 and 40 mg/day E_4 dose groups and a 2 mg/day E_2 -valerate control group all administered for 28 days, the effects of E_4 on vaginal cytology and endometrial thickness were investigated (unpublished data). In this study 2 mg/day E_4 for 28 days was as effective as 2 mg/day E_2 -valerate in shifting from mainly parabasal cells towards a high percentage intermediate and superficial cells, indicating estrogenic activity of E_4 . Proliferation of the endometrium with 2 mg/day E_4 was less than with 2 mg/day E_2 -valerate, but 10 mg/day E_4 had a stronger effect.

From these data it can be concluded that E_4 may be suitable for the treatment of urogenital atrophy and the accompanying clinical complaints such as vaginal dryness and dyspareunia in estrogen deficient women. Since E_4 induces proliferation of the endometrium, in women with a uterus, protection against endometrial hyperplasia and cancer may be required, depending on the optimal E_4 dose and the endometrial proliferation induced by that dose.

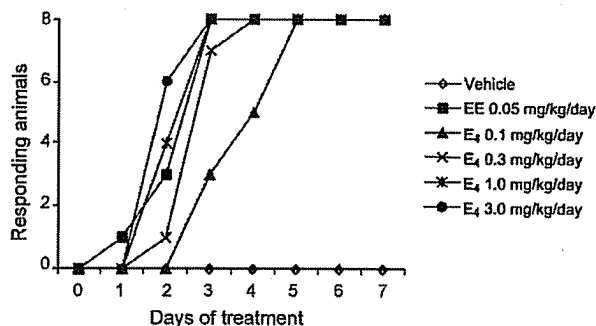


Fig. 2. Number of animals with vaginal cornification over a 7-day treatment period with orally administered estetrol (E_4 : 0.1, 0.3, 1.0 or 3.0 mg/(kg day)), ethinylestradiol (EE; 0.05 mg/(kg day)), or vehicle.

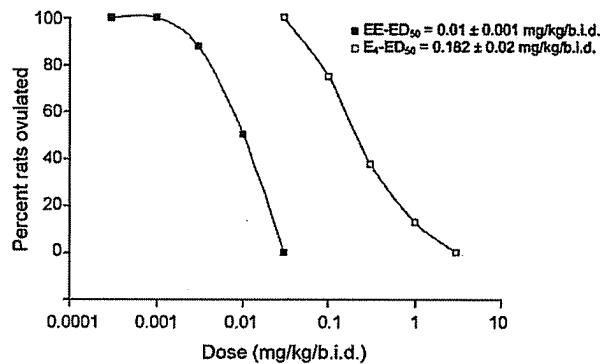


Fig. 3. Estimation of ED₅₀ for ovulation inhibition in 4-day cycling rats treated twice daily with the indicated oral doses of ethinylestradiol (EE) or estriol (E₄).

4. Contraception

The effects of E₄ on ovulation inhibition was studied in regularly cycling female rats [22,23]. The animals were treated orally twice daily for 4 consecutive days, starting on the day of estrus, with E₄ (0.03, 0.1, 0.3, 1.0 or 3.0 mg/kg), with the comparator EE (0.0003, 0.001, 0.003, 0.01 or 0.03 mg/kg) or with vehicle control. The primary endpoint was the number of ovulated oocytes in the genital tract. Ovulation was inhibited with E₄ at the twice-daily dose of 0.3 mg/kg and the higher doses ($p < 0.05$). Ovulation was also significantly inhibited ($p < 0.05$) by twice-daily administration of the comparator EE at the highest dose (0.03 mg/kg). The ED₅₀ of the dose response curves of EE and E₄ shows that E₄ is about 18 times less potent compared to EE [22] (Fig. 3).

In addition, the effect on plasma levels of gonadotrophins (LH and FSH) was studied in a single rising dose pharmacokinetics study in healthy postmenopausal women with administration of single doses of 0.1, 1, 10 and 100 mg E₄ [17]. LH levels were suppressed in a dose-dependent manner and a profound and sustained inhibition of FSH levels was observed, lasting over 7 days in the 100 mg dose group (FSH was only measured in the 100 mg dose group). In a multiple rising dose study in healthy postmenopausal women with 2, 10, 20 and 40 mg/day E₄ dose groups and a 2 mg/day E₂-valerate control group administered for 28 days, both FSH and LH levels decreased dose-dependently (Fig. 4). From these data it was concluded that E₄ has a profound central inhibitory and dose-dependent effect on gonadotrophins, expected to contribute to the contraceptive effect of E₄. This was further investigated in a phase IIA clinical trial in premenopausal women with proven ovulation

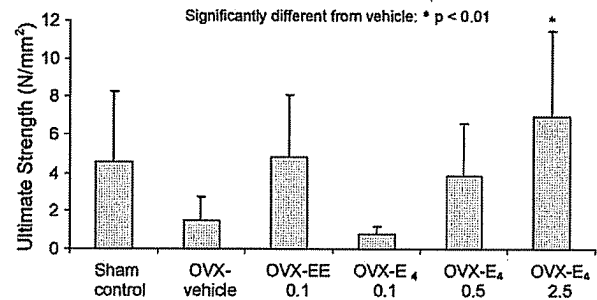


Fig. 5. Mean (\pm S.D.) ultimate strength (N/mm²) at the distal femora after 4 weeks of once-daily treatment with estriol (E₄; 0.1, 0.5 or 2.5 mg/(kg day)) or ethinylestradiol (EE; 0.1 mg/(kg day)) compared to vehicle in ovariectomised (OVX) rats and sham-operated controls.

in the previous cycle. In this study four treatment groups were included: a 10 mg E₄ only group, a 20 mg E₄ only group, a 20 mg E₄/150 mcg desogestrel group and a 20 mg E₄/200 mcg progesterone group. All treatments were for 28 days. In the E₄/desogestrel group ovulation was inhibited in all women, while in the 10 and 20 mg E₄ only groups in one-third and two-third of the cycles ovulation was inhibited respectively.

With both the 10 and 20 mg E₄ only doses no breakthrough bleeding or spotting occurred, while in the E₄/desogestrel group bleeding was acceptable.

In summary, based on the available data, it can be concluded that E₄ seems suitable to replace EE in combined oral contraceptives.

5. Osteoporosis

In ovariectomised (OVX) rats the bone-sparing effect of oral E₄ was compared to that of EE [16]. Once-daily oral treatment with a dose of 0.1, 0.5, or 2.5 mg/(kg day) of E₄ or by 0.1 mg/(kg day) of EE as positive control was given for 4 weeks. In this study the following parameters were assessed: (i) serum osteocalcin, (ii) bone mineral density (BMD), bone mineral content (BMC) and bone mineral area (BMA) of lumbar vertebrae L3–L6, (iii) peripheral quantitative computed tomography (pQCT) of the left tibiae and (iiii) the biomechanical properties (strength) of the distal femora.

In this rat model E₄ was able to significantly inhibit the OVX-related increases in osteocalcin levels, BMD and BMC, and bone strength (Fig. 5) in a dose-dependent manner. The relative potency of the highest dose of E₄ of 2.5 mg/(kg day) was comparable to the 0.1 mg/(kg day) EE dose, used as positive control. From these data it can be concluded that oral administration of E₄ conveys dose-

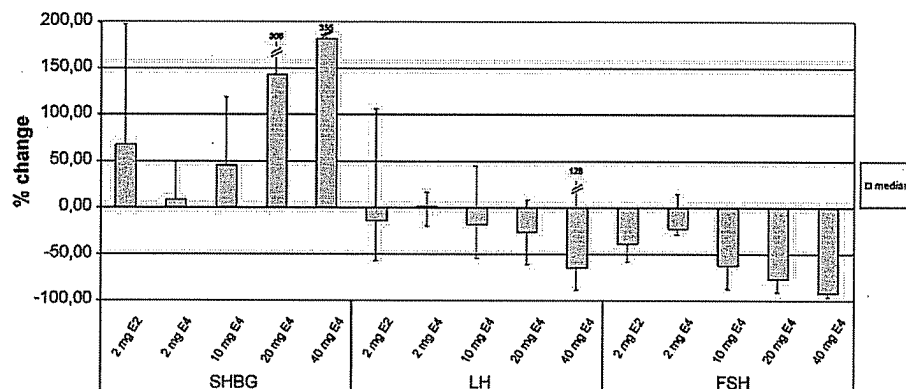


Fig. 4. The percentage change in plasma levels of luteinising hormone (LH), follicle stimulating hormone (FSH) and sex hormone binding globulin (SHBG) after administration of 2, 10, 20 and 40 mg/day estriol (E₄) and 2 mg/day estradiol-valerate (E₂) for 28 days.

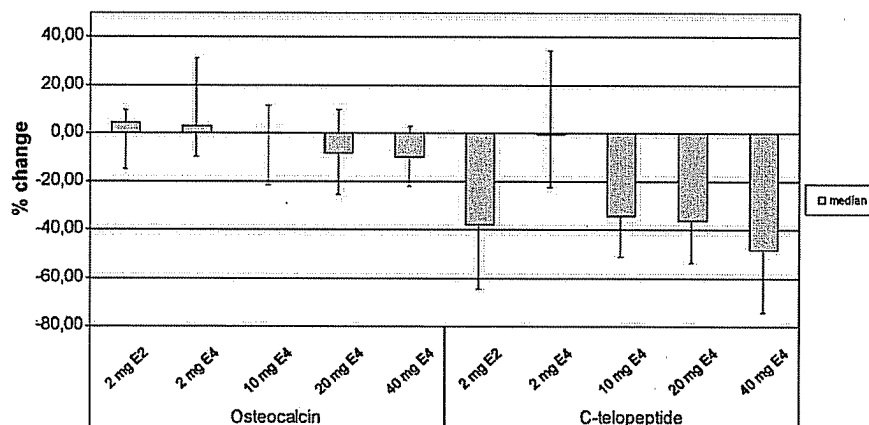


Fig. 6. The median percentage change in plasma levels of osteocalcin and C-telopeptide after administration of 2, 10, 20 and 40 mg/day estradiol (E₂) and 2 mg/day estradiol-valerate (E₄) for 28 days.

dependent bone-sparing effects of high quality bone in estrogen depleted OVX rats [16].

In the multiple rising dose study with E₄ in postmenopausal women a dose-dependent decrease of both the marker of bone resorption C-telopeptide and the marker of bone formation osteocalcin was observed (Fig. 6). At the higher dose levels of E₄ (20 and 40 mg) the inhibitory effect on bone formation was only 10%, whereas bone resorption was suppressed by 35–50%. This suggests uncoupling of bone metabolism with a preference for bone formation. These data indicate that E₄ may be suitable as drug for the prevention of osteoporosis in postmenopausal women. It may also be worthwhile to investigate the potency of E₄ to treat osteoporosis and osteoporotic fractures.

6. Breast cancer

Two prevention studies and one intervention study with E₄ were performed in a rat breast cancer model, in which the animals were treated with DMBA (7,12 dimethylbenz(a)anthracene) to develop estrogen-responsive breast tumors [24]. In the prevention studies the effect on the number and size of the tumors was investigated of oral doses of E₄ in a dose range of 0.5–3.0 mg/kg. The intervention study used oral doses of 1, 3 and 10 mg/kg E₄. As reference compound the anti-estrogen tamoxifen (TAM) was used in all three studies. Ovariectomy and EE at doses pharmacologically equipotent to E₄ acted as control treatments in one prevention study and in the intervention study.

A dose-dependent reduction in the number and size of tumors was seen when DMBA induced rats were co-treated with E₄ for 8 weeks, and this effect appeared to be equally effective as TAM treatment or OVX and was not seen with EE. Administration of E₄ to rats in which tumors had already been developed resulted in a significant decrease in the number and size of tumors after 4 weeks. This decrease was dose-dependent, comparable to the animals treated with TAM, and at high dose levels E₄ was as effective as OVX (Fig. 7) [24]. It was concluded that the growth of chemically induced mammary tumors is dose-dependently reduced by E₄ in female rats and in addition has the potential to reduce also dose-dependently the number and size of already present mammary tumors. Since in these DMBA studies no blood levels of hormones (E₂, E₄, and gonadotrophins) were measured, the effect of E₄ observed may be explained by two different mechanisms. Either there is an antagonistic effect of E₄ in the presence of E₂, or E₄ suppresses gonadotrophins and thereby E₂. In the first case the effect of E₄ may be explained by antagonism at receptor level. In the second

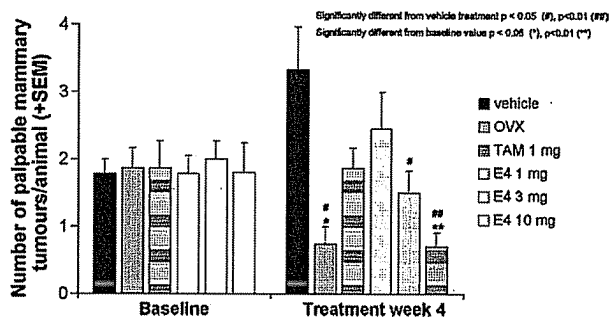


Fig. 7. Intervention study. Mammary tumor count per animal (+S.E.M.) at baseline and 4 weeks after ovariectomy (OVX) or daily oral treatment with vehicle, tamoxifen (TAM) or estradiol (E₄).

case the effect would result from suppression of E₂, comparable to ovariectomy. Whatever the case may be, the data show that a high dose of E₄ does not stimulate tumor growth. In case E₄ would suppress E₂ the control EE would possibly do the same. However, no reduction in the number and size of tumors was seen in EE-treated animals in contrast with E₄.

Based on this estrogen antagonistic effect, E₄ seems to be a suitable candidate for the treatment of HRT in women with (a history of) breast cancer, either for spontaneous climacteric symptoms or for symptoms induced by treatment of breast cancer with aromatase inhibitors or estrogen antagonists such as TAM. In addition E₄ may also be effective for the treatment of breast cancer itself. Currently a phase II neo-adjuvant study is ongoing investigating the effects of E₄ on proliferative and apoptotic markers in patients with breast cancer.

7. Conclusions

Estetrol is a steroid synthesized exclusively by the human fetal liver during pregnancy. After its discovery in 1965 basic research on E₄ was performed until about 1984. At that time E₄ was considered to be a weak estrogen and interest in this steroid disappeared. However, recently it has been shown that E₄ has a high and dose-related oral bioavailability in the rat [16] and the human [17], does not bind to SHBG [19] and has a long elimination half-life in both rat [16] and human [17], allowing its use as an oral once-a-day drug.

In well validated and predictive rat models E₄ behaves as an estrogen agonist in all tissues investigated, i.e. bone [16], vagina [21], myometrium [21], endometrium [21] and brain (hot flush [20]

and ovulation inhibition [20,21]), except for breast tumor tissue where this steroid acts as an estrogen antagonist in the presence of E₂ [24].

Estetrol may be useful for a series of potential clinical applications including hormone replacement therapy in women, especially for the treatment of vaginal atrophy and hot flushes, as estrogenic component in oral contraceptives, for the prevention and treatment of osteoporosis, and E₄ might even be suitable for prevention or treatment of breast cancer. These potential applications will be further explored in clinical trials.

Conflict of interest

MV is shareholder and employee of Pantarhei Bioscience, the company developing estetrol; HJTBC is CEO and shareholder of Pantarhei Bioscience.

References

- [1] A.A. Hagen, M. Barr, E. Diczfalussy, Metabolism of 17b-oestradiol-4-14C in early infancy, *Acta Endocrinol.* 49 (1965) 207–220.
- [2] G. Zucchini, B.P. Lisboa, E. Simonitsch, L. Roth, A.A. Hagen, E. Diczfalussy, Isolation of 15a hydroxyl-oestriol from pregnancy urine and from the urine of newborn infants, *Acta Endocrinol.* 56 (1967) 413–423.
- [3] J. Schwes, G. Eriksson, N. Wqvist, E. Diczfalussy, 15a-Hydroxylation: a new pathway of estrogen metabolism in the human fetus and newborn, *Biochim. Biophys. Acta* 100 (1965) 313–316.
- [4] J. Schwes, M. Govaerts-Videtsky, N. Wqvist, E. Diczfalussy, Metabolism of oestrone sulphate by the previsible human foetus, *Acta Endocrinol.* 50 (1965) 597–610.
- [5] S. Mancuso, G. Benagiano, S. Dell'Acqua, M. Shapiro, N. Wqvist, E. Diczfalussy, Studies on the metabolism of C-19 steroids in the human foeto-placental unit, *Acta Endocrinol.* 57 (1968) 208–227.
- [6] J. Heikkilä, H. Adlercreutz, A method for the determination of urinary 15a-hydroxyestriol and estriol, *J. Steroid Biochem.* 1 (1970) 243–253.
- [7] J. Heikkilä, Excretion of 15a-hydroxyestriol and estriol in maternal urine during normal pregnancy, *J. Steroid Biochem.* 2 (1971) 83–93.
- [8] C.F. Holinka, E. Diczfalussy, H.J.T. Coelingh Bennink, Estetrol: a unique steroid in human pregnancy, *J. Steroid Biochem. Mol. Biol.* 110 (2008) 138–143.
- [9] F. Coelingh Bennink, C.F. Holinka, M. Visser, H.J.T. Coelingh Bennink, Maternal and fetal estetrol levels during pregnancy, *Climacteric* 11 (Suppl. 1) (2008) 69–72.
- [10] J. Heikkilä, T. Luukkainen, Urinary excretion of estriol and 15a-hydroxyestriol in complicated pregnancies, *Am. J. Obstet. Gynecol.* 110 (1971) 509–521.
- [11] D. Tulchinsky, F.D. Frigoletto, K.J. Ryan, J. Fishman, Plasma estetrol as an index of fetal well-being, *J. Clin. Endocrinol. Metab.* 40 (1975) 560–567.
- [12] A.D. Notation, G.E. Tagatz, Unconjugated estriol and 15a-hydroxyestriol in complicated pregnancies, *Am. J. Obstet. Gynecol.* 128 (1977) 747–756.
- [13] N. Kundu, M. Grant, Radioimmunoassay of 15a-hydroxyestriol (estetrol) in pregnancy serum, *Steroids* 27 (1976) 785–796.
- [14] N. Kundu, M. Wachs, G.B. Iverson, L.P. Petersen, Comparison of serum unconjugated estriol and estetrol in normal and complicated pregnancies, *Obstet. Gynecol.* 58 (1981) 276–281.
- [15] H.J.T. Coelingh Bennink, C.F. Holinka, E. Diczfalussy, Estetrol review: profile and potential clinical applications, *Climacteric* 11 (Suppl. 1) (2008) 47–58.
- [16] H.J.T. Coelingh Bennink, A.M. Heegaard, M. Visser, C.F. Holinka, C. Christiansen, Oral bioavailability and bone-sparing effects of estetrol in an osteoporosis model, *Climacteric* 11 (Suppl. 1) (2008) 2–14.
- [17] M. Visser, C.F. Holinka, H.J.T. Coelingh Bennink, First human exposure to exogenous oral estetrol in early postmenopausal women, *Climacteric* 11 (Suppl. 1) (2008) 31–40.
- [18] M. Visser, J.-M. Foidart, H.J.T. Coelingh Bennink, In vitro effects of estetrol on receptor binding, drug targets and human liver cell metabolism, *Climacteric* 11 (Suppl. 1) (2008) 64–68.
- [19] G. Hammond, K. Hogeveen, M. Visser, H.J.T. Coelingh Bennink, Estetrol does not bind sex hormone binding globulin or increase its production by human HepG2 cells, *Climacteric* 11 (Suppl. 1) (2008) 41–46.
- [20] C.F. Holinka, M. Brincat, H.J.T. Coelingh Bennink, Preventive effect of oral estetrol in a menopausal hot flush model, *Climacteric* 11 (Suppl. 1) (2008) 15–21.
- [21] A.M. Heegaard, C.F. Holinka, P. Kenemans, H.J.T. Coelingh Bennink, Estrogenic uterovaginal effects of oral estetrol in the modified Allen-Doisy test, *Climacteric* 11 (Suppl. 1) (2008) 22–28.
- [22] H.J.T. Coelingh Bennink, S. Skouby, P. Bouchard, C.F. Holinka, Ovulation inhibition by estetrol in an in vivo model, *Contraception* 77 (3) (2008) 186–190.
- [23] H.J.T. Coelingh Bennink, S. Skouby, P. Bouchard, C.F. Holinka, Ovulation inhibition by estetrol in an in vivo model, *Climacteric* 11 (Suppl. 1) (2008) 30 (a summary from *Contraception* 77(3) (2008) 186–190).
- [24] H.J.T. Coelingh Bennink, C. Singer, T. Simoncini, A. Genazzani, E. Kubista, Estetrol, a pregnancy-specific human steroid, prevents and suppresses mammary tumor growth in a rat model, *Climacteric* 11 (Suppl. 1) (2008) 29.